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RESEARCH ARTICLE

Validated Chromatographic methods for estimation of Solanine in *Mahamrutyunjaya rasa*

Pallavi Rai[1*], Rahul Kaushik[1], Sadhana J. Rajput[2]

ABSTRACT

Introduction: *Mahamrutyunjaya rasa* is a herbomineral ayurvedic formulation used for various indications like fever, pain, cardiac arrhythmia, etc. It contains *Solanum indicum* as one of the ingredients having anti-inflammatory, diuretic, and diaphoretic properties. Solanine is one of the active biomarkers for *Solanum indicum*. The therapeutic window of Solanine is very narrow, with the LD_{50} value of 3–6 mg/kg body weight in humans.

Objective: Estimation of such biomarkers is very significant to avoid any adverse events due to the administration of ayurvedic preparations containing Solanine. In the present study, two simple, sensitive, reliable chromatographic techniques have been developed and validated for the estimation of Solanine in *Mahamrutyunjaya rasa*.

Materials and Methods: High-performance liquid chromatography (HPLC) separation of Solanine was performed on an reversed phase C-18 column (250 mm 9 4.6 mm ID, 5 lm particle size), with isocratic elution using a mixture of Tris buffer (10mM, pH 6.00): Acetonitrile(60:40, v/v) at a flow rate of 1 mL/min with UV detection at 218 nm for solanine. High-performance thin-layer chromatography (HPTLC) separation was done on Silica gel 60 F254 pre-coated plates using chloroform: Methanol: Ammonia (7:3:0.5 v/v). The densitometric scanning was performed at 500 nm. The developed methods were validated for linearity, limit of detection (LoD), limit of quantitation (LoQ), accuracy, precision, and specificity as per ICH guidelines.

Results: Solanine was eluted at 4.43 ± 0.1 min and established a linearity range of 1 – 100 µg/ml (r^2 = 0.9992). In the HPTLC method, the Rf value of Solanine was 0.05 in a linearity range of 1600-4800 ng/ml.

Conclusion: Reliable, rapid, simple, and sensitive chromatographic methods were developed for the quantification of Solanine in *Mahamrutyunjaya Rasa*.

Keywords: Ankylosing, Spondylarthropathies, Spondylitis, Thalidomide, Tumor necrosis factor-alpha.

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INTRODUCTION

Quality control and standardization of ayurvedic medicines have proven to be the prime key for their acceptance in the worldwide market.[1] Lack of clinical evidence and regulatory control of these medicines has become a bottleneck in creating a commercial market and alternative options for the treatment of many chronic diseases.[2] Ayurvedic medicines often contain alkaloid rich crude drugs that have a very narrow therapeutic index.[3] Thus, if simple, reliable, sensitive, and reproducible analytical methods are developed for estimation of the bio-markers of the crude drugs and formulations, it will help in determining the exact quality of the finished products.

Mahamrutyunjaya Rasa (MHR) is a herbal-mineral formulation comprising of *Aconitum ferox, Solanum indicum, Piper nigrum*, and *Piper longum*. It is used for the treatment of cardiac problems. *Bhaishjaya ratnavali* (*A.S.S Rasa rasayana prakarana*)[4] records the formula of an MHR tablet which contains one part of processed *Aconitum ferox*, one part of *Solanum indicum*, one part of *Piper nigrum* and one part of *Piper longum* which are powdered and sieved through a 100 mesh sieve. It is then mixed with one part purified sulfur, one part purified sodium metaborate and two parts of purified cinnabar. The Solanum roots also contain toxic glycoalkaloids, one of which is Solanine.[5,6]

Various chromatographic methods have been developed and reported, for example, HPTLC methods for the analysis of solanine in potatoes.[7,8] Isolation and characterization studies by preparative TLC has also been reported.[9] An LC method for estimation of Solanine in potato cultivars has also been reported.[10] RPLC method has been reported for simultaneous estimation with piperine and aconitine, which is time-consuming.[11] However, no validated methods are available for the determination of only Solanine in *Solanum indicum* roots and ayurvedic formulations.

In the current work, we investigated the effects of various chromatographic parameters and successfully

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developed as well as validated HPLC and HPTLC method for the quantitative determination of Solanine in ayurvedic medicine, MHR.

MATERIALS AND METHODS

Chemicals and Reagents

The standard Solanine (97% from potato sprouts) was purchased from Sigma Aldrich Pvt. Acetonitrile and methanol were of HPLC grade. Potassium dihydrogen phosphate (KH_2PO_4) and 85% *ortho*-phosphoric acid (H_3PO_4) of analytical-reagent grade were also purchased from Qualigens, Mumbai. Triple distilled water was used for the study. Chloroform, methanol, and ammonia of analytical grade were purchased from Qualigens (Mumbai). Draggendorff‟s reagent was prepared as per the reported method.[12] All the other solvents and reagents used were of analytical grade and were filtered through a 0.2 μm Ultipor®Nylon 66 membrane filter (Pall Life Sciences, USA) prior to use.

Preparation of Standard Solutions

Standard stock solutions of solanine (1000 μg/mL) were prepared by dissolving 10 mg of pure solanine in 10 mL methanol. Appropriate and accurate volume aliquots of the stock solutions were transferred to 10 mL calibrated flasks and diluted to volume with methanol in the range of 10-100 μg/mL.

HPLC method

Preparation of Sample Solutions

Twenty tablets of all the three formulations were powdered, and about 1gm, each of the three formulations was accurately weighed and extracted in 25 mL of 0.1N HCl by sonication for 10 minutes at room temperature, which was then fractionated with 10 mL ethyl acetate thrice to remove the non-alkaloid components. The acidic aqueous solutions were basified using ammonia to pH 11 and further extracted with 10 mL chloroform thrice. Chloroform was evaporated under reduced pressure. The residue was dissolved in methanol by sonication, and further dilutions were made in acetonitrile.

Analytical Conditions

The analysis was isocratic at 1.0 mL/min flow rate with Tris buffer (10 mM, pH 6.00): acetonitrile (60:40 v/v) as the mobile phase. The mobile phase was prepared freshly every day. The mobile phase was filtered through a 0.2 μm membrane filter to remove any particulate matter, mixed and degassed by sonication before use. The absorbance of solanine was good at 218 nm, and further, it was free from any interference. Hence, the eluted peak was detected at 218 nm. The sensitivity of the detector was set at 0.01 AUFS. Before injecting solutions, the column was equilibrated for at least 60 minutes, with the mobile phase flowing through the system. Each solution was injected in triplicate, and the relative standard deviation (RSD) was required to remain below 1.0% on a peak area basis.

Optimization of Chromatographic Conditions

Chromatographic separations are significantly affected by the mobile phase conditions, such as the type and composition of the organic modifiers. Therefore, before selecting the conditions for the optimization, many preliminary trials were conducted with different combinations of different organic solvents and buffers at various pH, mobile phase compositions, and flow rate to check the retention time, shape, resolution, and other system suitability parameters of all the peaks.

To achieve an optimum separation, the following conditions were studied: (i) Mobile phase pH varied at 5,6, and 7 keeping the composition of Tris buffer (10 mM, pH 6.00): acetonitrile (60:40 v/v) and flow rate of 1.0 ml/min fixed. (ii) Mobile phase composition varied at 55:45, 60:40 and 65:35 (v/v) with pH and flow rate kept constant at 6 and 1.0 mL/min, respectively. (iii) The flow rate was varied (0.8, 1.0, and 1.2 mL/min) with mobile phase composition and pH maintained at 60:40 (v/v) and 6, respectively. The effects of all these three factors were systematically addressed on system suitability parameters such as resolution, theoretical plates, retention time, capacity factor, separation factor, asymmetry, and HETP, etc.

All mobile phases used in the optimization study were prepared by mixing the buffer system with the organic solvent in the desired proportions.

Method Validation

To verify that the proposed method is applicable to formulation analysis, validation was performed as per the ICH Guidelines.[13]

Calibration curve (linearity)

Seven different concentrations of solanine were analyzed, and their calibration curve was constructed in the specified concentration range (1–100 μg/mL). The calibration plots were generated by replicate analysis (n = 3) at all concentration levels, and the linear relationship was evaluated using the least square method within Microsoft Excel® program.

Repeatability, Precision, and Stability

The injection repeatability was determined by the analysis of six continuous injections using the same sample, while the analysis repeatability was examined by the injection of six different samples prepared by the same procedure. The standard solution (10, 40, 100 μg/mL)

was used for the test of injection repeatability and analysis repeatability.

The instrument precision was examined by performing the intra-day, and inter-day assays of six replicate injections of the standard solutions at three concentration levels (10, 40, 100 μg/mL). The intra-day assay precision was performed with an interval of 4 hours in 1 day, while the inter-day assay precision was performed over 6 days.

Limit of Detection (LoD) and Limit of Quantification (LoQ)

LOD and LOQ were determined by *kSD*/*s* where *k* is a constant (3 for LoD and 10 for LoQ), *SD* is the standard deviation of the analytical signal, and *s* is the slope of the concentration/response graph.

Robustness

Robustness of the proposed method was evaluated by keeping the chromatographic conditions constant, except for the followings changes:

- Detection wavelength: Changed from 218 nm to 216 nm and 220 nm
- Column: Using another column (Hypersil ODS, particle size 5 μm; 250 mm X 4.6 mm ID)
- Solvent Brand: Acetonitrile and methanol supplied by Spectrochem Pvt Ltd. (Mumbai, India) and Rankem (Mumbai, India).

The standard solution was injected 6 times for each change. System suitability parameters like resolution, peak asymmetry, theoretical plates, capacity factor, and RSD were calculated for each peak. Recoveries and % RSDs were calculated for each component during each change.

Accuracy

The accuracy of the method was determined by calculating the recoveries of solanine by the method of standard additions. Known amounts of standard (80, 100, and 120%) was added to the pre-analyzed sample solution, and the amount of the standard was estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

HPTLC Method

Preparation of Sample Solutions

The methanol extract of the powdered formulation was prepared by sonication for 20 minutes. The extract was concentrated and evaporated under a vacuum. A total of 100 mg of dry extract was weighed and dispersed in methanol in a 10 mL volumetric flask. The volume was made up to 10 mL.

Application of test samples

The samples were spotted in the form of bands of width 6 mm on the pre-coated silica gel G plates. The plates were pre-washed by methanol and activated at 110°C for 5 minutes before chromatography. A constant application rate of 0.1 μL/s were employed, and space between two bands was 6 mm. For the calibration curve of solanine, the stock solution was used. Different volumes from 1.6–4.8 μl of stock solution were applied, which gave different concentrations of 1600-4800 ng per spot, respectively.

Development

The mobile phase consisted of chloroform: methanol: ammonia (7:3:0.5 v/v) Linear ascending development was carried out in a trough chamber saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 15 minutes at room temperature. The length of the chromatogram run was 85 mm. Subsequent to the development, TLC plates were dried in a current of air with the help of an air-dryer.

Detection

Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. The slit dimension was kept at 5 mm x 0.45 mm, and 10 mm/s scanning speed was employed. The monochromator bandwidth was set at 20 nm with K 320 cut off filter, each track was scanned thrice, and baseline correction was used. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. The plate was sprayed with Dragendorffs reagent immediately scanned and quantified at 500 nm using the Camag TLC Scanner-3. Data of the peak area of each band were recorded. A calibration curve was obtained by plotting peak area Vs. concentration and peak height Vs. concentration of solanine. Spectra of the samples and standard solanine were matched.

Method Validation

Calibration Curve of Solanine.

A stock solution of solanine (1000 μg/mL) was prepared in methanol, and bands in the range of 1600–4800 ng per spot were applied. The drug was spotted in duplicate on the TLC plate to obtain concentrations of 1600–4800 ng per spot of solanine. The data of peak height/ area versus drug concentration were treated by linear least-square regression.

Precision

The intra-day and inter-day variation for the determination of solanine was carried out at two different concentrations levels 1600 and 4800 ng per spot.

Robustness of the Method

By introducing small changes in the mobile phase composition, the effects on the results were examined.

Mobile phases having different compositions were tried at two different concentration levels of 1600 and 4800 ng per spot.

Limit of Detection and Limit of Quantitation

To estimate the LoD and LoQ, blank methanol was spotted six times. The signal-to-noise ratio was determined. An LoD was considered as 3:1 and LOQ as 10:1. The LOD and LoQ were experimentally verified by diluting known concentrations of solanine until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations.

Recovery Studies

The analyzed samples were spiked with an extra 80, 100, and 120% of the standard solanine and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulations.

RESULTS AND DISCUSSION

High-performance Liquid Chromatography (HPLC) Method

Optimization of chromatographic conditions

Different parameters for chromatographic separation like different stationary phases (i.e., C18 and C8 columns), pH, and mobile phase composition were optimized to achieve maximum separation between solanine and other components present in the polyherbal formulation. The very sharp and symmetric peak of solanine was observed when a Phenomenex Luna C18 (250 mm × 4.6 mm, 5 µm) column was employed. The pH 6 gave sufficient separation as well as symmetric peak shape. The final composition of the mobile phase was determined by varying the buffer: the organic modifier ratio to give the best results. Several studies were carried out by decreasing the percentage of acetonitrile from 60–40%, until satisfactory resolution was obtained. A proportion of acetonitrile (40%) resulted in smoothening of baseline and complete separation of solanine from its degradation products. Finally, the mobile phase comprising of Tris buffer (10 mM; pH 6.0 adjusted with 1% Triethylamine)-acetonitrile (60: 40 v/v) showed good resolution, good peak symmetry, was used to formulation samples (Figure 1).

The detection wavelength was decided to be 218 nm from the UV scan in the range of 200–00 nm.

Validation

Calibration curve (linearity)

The calibration curves (n=3) constructed for solanine was linear over the concentration range of 1-100 µg/mL. Peak area of the marker was plotted versus the concentration and linear regression analysis performed on the resultant curve. The coefficients of determination 0.999 with %RSD values ranging from 0.5–2% across the concentration range studied were obtained following linear regression analysis (Figure 2, Table 1).

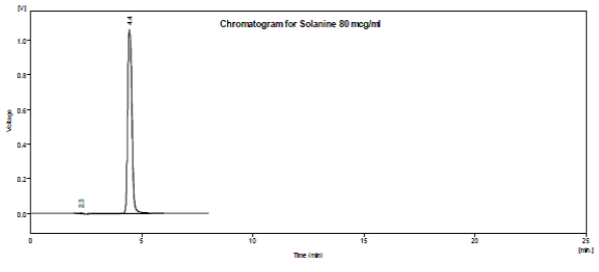

Figure 1: Representative HPLC chromatograms of solanine standard.

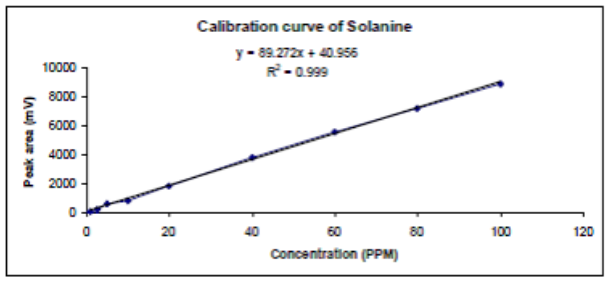

Figure 2: Calibration curve of solanine (1-100 µg/mL)

Table 1: Linear regression data for the calibration curves (n = 3) of HPLC method

S.N.	*Parameter*	*Values*
1	Retention time, min	4.43 + 0.1
2	Detection wavelength, nm	218 nm
3	LOD, µg/mL	0.14
4	LOQ, µg/mL	0.345
5	Linearity range	1-100 µg/mL
6	Coefficient of determination (height)	0.9992
7	Coefficient of determination (area)	0.9996
8	Regression equation (height)	Y = 4.2316x – 0.0965
9	Regression equation (area)	Y = 89.272x + 40.956
10	Slope (height)	4.2316 + 0.13
11	Slope (area)	89.272 + 1.176
12	Intercept (height)	0.0965 + 0.05
13	Intercept (area)	40.956 + 1.04

Repeatability, Precision, and Stability (Table 2)

Injection repeatability: The calculated % RSDs of the peak areas was less than 2% at each of the three concentration levels.

Analysis repeatability: The RSD values for analysis repeatability were less than 2% both for retention time and peak area.

Instrument precision: The precision result of the solution at medium concentration is presented in Table 3, and it was shown that the %RSD values of retention time were less than 1%, while the %RSD values of peak area were less than 2% both for intra-day assay and inter-day assay precision (Intra 4 hour six injections, inter 6 days). For the stability test, the same sample was analyzed within 24 hours at room temperature, and the solution was found to be stable (RSD values of the retention time and peak area were both less than 3%).

Limit of Detection and Limit of Quantification

The LoD and LoQ were found to be 0.14 and 0.345 µg/mL for Solanine.

Specificity

Satisfactory results were obtained, indicating the high specificity of the proposed method for the determination of solanine in the formulations.

Robustness

Table 4 shows the mean obtained (n=6) for each factor studied, indicating that the selected factors remained unaffected by small variations of these parameters. The recovery obtained individually, and the mean was between 98% and 101% Solanine. Therefore, it can be concluded that the method is consistent for detection wavelength, selected column, and solvent brand.

System Suitability

A system suitability test was performed to evaluate the chromatographic parameters (capacity factor, separation factor, column efficiency, number of theoretical plates, HETP asymmetry of the peaks and resolution between two consecutive peaks) before the validation runs (Table 5). Three replicate injections of the standard solution and three injections of the solution prepared for the specificity procedure were used.

Accuracy

As shown in Table 6, the recovery of the investigated components ranged from 98–101%, and their %RSD values were all less than 2%. It was known from recovery tests that the developed method manifested the reliability and accuracy for the measurement of these components.

Table 2: Repeatability of the developed HPLC method (n = 6)

Solanine (µg/mL)	Retention time (min)				Peak area (mVs)			
	Injection repeatability		Analysis repeatability		Injection repeatability		Analysis repeatability	
	Mean	%RSD	Mean	%RSD	Mean	%RSD	Mean	%RSD
10	4.43	0.28	4.42	0.14	79.08	1.08	80.13	1.66
40	4.41	0.34	4.43	0.22	3870.89	1.67	3865.82	1.25
100	4.42	0.27	4.43	0.29	8867.98	1.29	8860.15	1.73

Table 3: Intra-day and inter-day precision of the developed HPLC method (n = 6).

Solanine (µg/mL)	Intra-day precision				Inter-day precision			
	Mean area	S.D.	%RSD	S.E.	Mean area	S.D.	%RSD	S.E.
10	79.08	2.12	1.98	2.34	80.67	1.76	1.93	2.32
40	3870.89	5.78	1.34	2.98	3886.12	6.78	1.90	4.09
100	8867.98	12.09	1.09	3.98	8872.31	15.65	1.98	5.13

Table 4: Robustness of HPLC method (n = 3)

Chromatographic change		
Factor	Level	Recovery, %
Wavelength, nm		
215	-1	98.73 ± 2.19
218	0	99.75 ± 1.27
221	1	99.76 ± 2.76
Column brand		
Phenomenex	1	99.42 ± 0.84
Hypersil	2	98.31 ±1.73
Acetonitrile brand		
Rankem	1	100.67 ± 1.23
Spectrochem	2	99.87 ± 1.45
Qualigens	3	98.43 ± 2.65

Table 5: System Suitability Parameters of HPLC method

Sr. No.	Parameter	Values
1	Retention time (min)	4.43
2	Area (mVs)	565.44
3	Capacity factor (k')	3.08
4	Efficiency/length (t.p./m)	514232
5	HETP (mm)	0.047
6	Resolution (Rs)	7.13
7	Asymmetry	1.10

Table 6: Recovery test (n = 3) of the HPLC method

Excess drug added to the analysis (%)	Theoretical content (µg)	Recovery (%)	% RSD
0	13.30	100.12	1.45
80	23.94	99.45	1.34
100	26.60	98.67	1.76
120	29.26	99.08	1.98

Applicability of the Developed Method in Formulations

The developed HPLC method was applied to the determination of solanine in the Ayurvedic formulations (FORM1, FORM2, FORM3), and the results are presented in Figure 3, Table 7. It was observed that the content of solanine varied in the three formulations, with the %RSD values of higher than 5 %, which would significantly influence the quality stability because it is one of the target toxic components for the quality control of MHR tablets.

High-performance Thin Layer Chromatography (HPTLC) Method

Development of the Optimum Mobile Phase

The TLC procedure was optimized with a view to develop an assay method for the estimation of Solanine. Both the pure drug and the formulation extract solution were spotted on the TLC plates and run in different solvent systems. Initially, chloroform: Methanol in varying ratios was tried. The mobile phase chloroform: methanol (7:3, v/v) gave good resolution, but Rf value was less. Also, the typical peak nature was missing because the spot was slightly diffused. The addition of 0.5 ml of 28% ammonia solution to the above mobile phase improved the spot characteristics and increased the Rf value to 0.05 ± 0.02 when densitometric scanning was performed at 500 nm after derivatization with dragendorff's reagent. Finally, the mobile phase consisting of Chloroform: Methanol: Ammonia (7:3:0.5 v/v/v) gave a sharp and symmetrical peak. Resolution between spots of standard and other components appeared better when TLC plates (pretreated with methanol and activated at 100°C for 5 min). Well-defined spots (compact dense spots) were obtained when the chamber was saturated with the mobile phase for 15 min at room temperature (Figure 4).

Validation of the Method

Linearity

The calibration graph was found to be linear that is the adherence of the system to Kubelka Munk theory[14] which was found over the concentration range of 1600–4800 ng/spot (r^2 = 0.99932). Linearity was evaluated by determining the standard working solution containing 400 µg/ml of solanine in triplicate. Peak area and concentration were subjected to least-square linear regression analysis to calculate the calibration equation

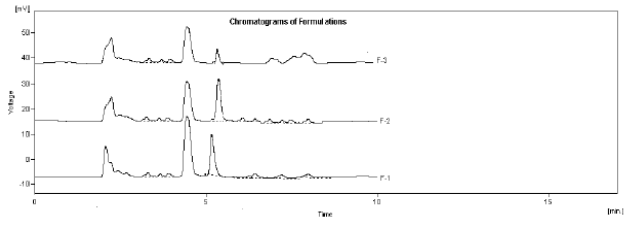

Figure 3: Chromatograms of different marketed MHR formulations (F-1, F-2, F-3)

Figure 4: HPTLC Chromatogram of Solanine Samples (1600-4800 ng/mL) and Samples U1-U3 of marketed MFR formulations at 366nm

Table 7: Contents of the solanine in three proprietary Ayurvedic medicines (n = 3) as per HPLC method

S.N.	Formulation	Solanine, µg/tablet
1	Form 1	0.13 ± 0.01
2	Form 2	0.145 ± 0.02
3	Form 3	0.078 ± 0.01

and correlation coefficients. The regression data showed a good linear relationship over the low concentration range of 1600–4800 ng/spot (Table 8). A high value of correlation coefficient validated the linearity of calibration graphs and adherence of the system to Kubelka Munk theory, and the SD for intercept value was less than 2. No significant difference was observed in the slopes of standard plots.

Precision

The repeatability of the sample application and measurement of peak area was expressed in terms of % RSD and found to be 1.84 and 1.67, respectively for six replicate determinations. The %RSD for intra-day and inter-day variation of solanine peak area at two different concentration levels 1600, 4800 ng/spot are shown in Table 9, respectively.

Robustness of the method

The standard deviation of peak areas was calculated for each parameter, and RSD was found to be less than 3%. The low values of % RSD, as shown in Table 10 indicated the robustness of the method.

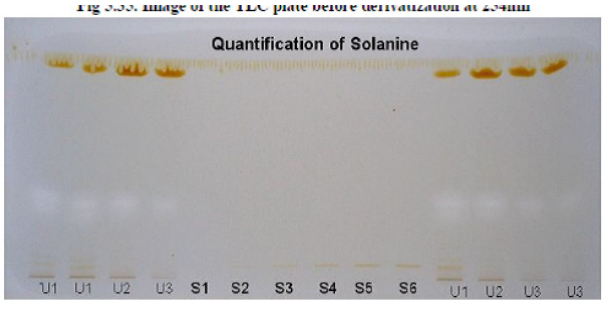

Figure 5: HPTLC Chromatogram of Solanine Samples (1600-4800 ng/mL) and Samples U1-U3 of marketed MFR formulations in Visible range.

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

The calibration plot in this study was plotted between amount of analyte versus average response (peak area) and the regression equation with a regression coefficient of 0.999 was obtained. Detection limit and quantification limit was calculated by the method as described earlier and found 50 ng and 100 ng, respectively, which indicates the adequate sensitivity of the method.

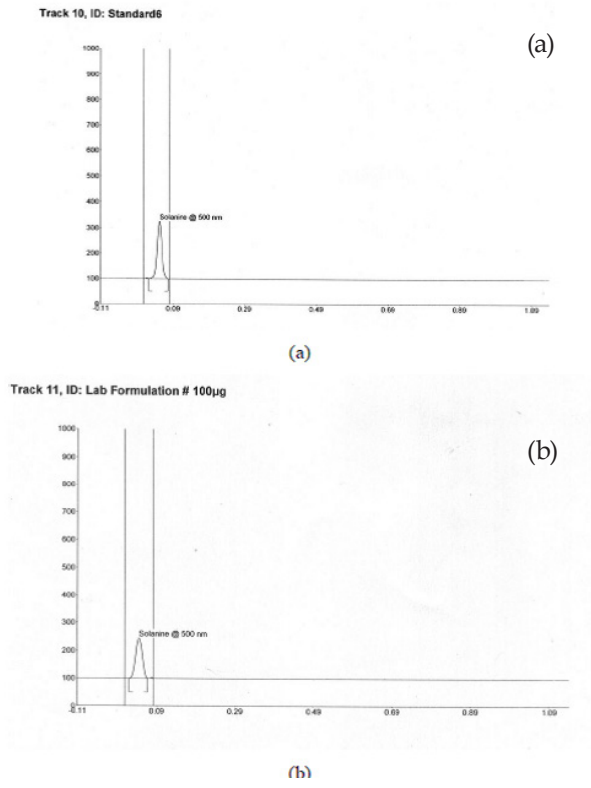

Figure 6: Chromatograms of Solanine (4800ng/mL) and MHR formulation

Table 8: Regression analysis for calibration plots. (n = 3)

S.N.	*Parameters*	*Values*
1	Linearity Range	1600 - 4800 ng/mL
2	Coefficient of determination (height)	0.99344
3	Coefficient of determination (area)	0.99242
4	Regression equation (height)	Y = 44.77x + 15.14
5	Regression equation (area)	Y = 552.3x – 16.6
6	Slope (height)	44.77 ± 1.02
7	Slope (area)	552.3 + 2.23
8	Intercept (height)	15.14 ± 0.83
9	Intercept (area)	16.6 ± 0.21

Table 9: Intra-day and Inter-day precision of HPTLC method. (n = 6)

	Intra-day precision		
Amount (ng/spot)	*Mean area*	*S.D.*	*%RSD*
1600	758.97	24.98	1.05
4800	2541.88	76.83	1.65
Amount (ng/spot)	Inter-day precision		
	Mean area	S.D.	%RSD
1600	760.32	38.52	1.23
4800	2545.40	82.66	1.57

Table 10: Robustness of the HPTLC method.

	Mobile Phase Composition %RSD	
Amount (ng/spot)	*Chloroform: Methanol: Ammonia (7:3:0.5 v/v)*	*Chloroform: Methanol: Ammonia (7.5:2.5:0.5 v/v)*
1600	1.76	1.94
4800	1.88	2.04

Table 11: Recovery studies of HPTLC method (n = 3)

Excess Drug added to the analyte (%)	*Theoretical Content (ng)*	*Recovery (%)*	*%RSD*
0	1600	99.45	1.63
80	2880	98.72	1.88
100	3200	98.34	2.23
120	3520	99.21	1.95

Table 12: Results of analysis of HPTLC method

S.N.	*Formulation*	*Solanine, µg/ tablet*
1	Form 1	0.11 ± 0.02
2	Form 2	0.158 ± 0.02
3	Form 3	0.070 ± 0.01

Specificity

The peak purity of solanine was assessed by comparing the spectra at peak start, peak apex, and peak end positions[15] of the spot, i.e., $r^2 = 0.9991$ and $r^2 = 0.9988$. Good correlation ($r^2 = 0.9996$) was also obtained between standard and sample spectra of solanine.

Accuracy

The proposed method when used for extraction and subsequent estimation of solanine from pharmaceutical dosage form after spiking with 80, 100 and 120% of additional drug afforded recovery of 98–100% as listed in Table 11

Spot stability

No decomposition was observed during spotting and development.

Analysis of the Marketed Formulation

A compact spot at R_f 0.05 was observed in the chromatogram of the drug samples extracted from tablets. There was no interference from the other components present in the formulation. The good performance of the method indicated the suitability of this method for routine analysis of solanine in the herbal dosage form.

CONCLUSION

Two different methods were developed and validated for the determination of Solanine using HPLC and HPTLC chromatographic instruments. The methods were found to be simple, sensitive, accurate, and precise and can be applied for the estimation of Solanine in ayurvedic formulations. Since proprietary ayurvedic medicines of Solanum species are progressively increasing[16-17] and showing therapeutic effects, methods for the assay is also the need of the hour[18] The above-reported methods can be used for the determination of Solanine in routine Quality control and thus prove to be safe for patient administration.

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CONFLICT OF INTEREST

Dr. Pallavi Rai, Mr. Rahul Kaushik, and Dr. S.J. Rajput declare no conflict of interest regarding the publication of this article.

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